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Dynamic relationships between body fat and circulating adipokine levels from adolescence to young adulthood: The Santiago Longitudinal Study

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ABSTRACT

Background and Aims: Adipose tissue secretes adipokines such as adiponectin and leptin, playing important roles in energy metabolism. The longitudinal associations between such adipokines and body fat accumulation have not been established, especially during adolescence and young adulthood and in diverse populations. The study aims to assess the longitudinal association between body fat measured with dual X-ray absorptiometry and plasma adipokines from adolescence to young adulthood.

Methods and Results: Among Hispanic/Latino participants (N=537) aged 16.8 (SD: 0.3) years of the Santiago Longitudinal Study, we implemented structural equation modeling to estimate the sex-specific associations between adiposity (body fat percent (BF%) and proportion of trunk fat (PTF)) and adipokines (adiponectin and leptin levels) during adolescence (16y) and these values after 6 years of follow-up (22y). In addition, we further investigated whether the associations differed by baseline insulin resistance (IR) status. We found evidence for associations between 16y BF% and 22y leptin levels (β(SE): 0.58(0.06) for females; 0.53(0.05) for males), between 16y PTF and 22y adiponectin levels (β(SE): -0.31(0.06) for females; -0.18(0.06) for males) and between 16y adiponectin levels and 22y BF% (β(SE): 0.12(0.04) for both females and males).

Conclusion: We observed dynamic relationships between adiposity and adipokines levels from late adolescence to young adulthood in a Hispanic/Latino population further demonstrating the importance of this period of the life course in the development of obesity.

INTRODUCTION

Obesity is a vast public health burden worldwide. Excessive body weight, even early in the life course, is critical in obesity-associated cardiometabolic disorders.[1,2] Adolescence is an especially dynamic period in terms of changes in body size and body composition, partially related to hormonal changes.[3] In particular, adipose tissue is actively functioning as a part of the endocrine system during puberty.[3] Adipose tissue secretes various signaling molecules, adipokines. Adiponectin and leptin are two major adipokines playing significant roles in energy metabolism.

Adiponectin is well-known for its protective roles against obesity-associated cardiometabolic disorders, including insulin-sensitizing, anti-inflammation, and anti-atherogenesis.[4,5] Leptin is a hunger hormone, but its levels are typically high among individuals with obesity due to increased fat mass and leptin resistance.[6] Correlations between these adipokines and body weight have been reported. Specifically, many studies find that adiponectin levels are inversely associated with adiposity[7-11] and leptin levels are positively associated with adiposity.[6,12,13] One limitation that hinders a conclusion to be drawn about the causal relationships between adipokines and adiposity is the cross-sectional design of these studies. Further, even among the longitudinal studies that have investigated the associations between adipokines and body weight or adiposity[14-26], only one direction of the association – i.e., either from baseline adipokine to follow-up adiposity or from baseline adiposity to follow-up adipokines – was considered. Yet, the relationship between specific adipokines and adiposity is not conclusive, and it is likely sensitive to life course effects.[14] Adipokines are signaling molecules that may affect body fat accumulation via the regulation of energy homeostasis.[27,28] At the same time, adipose tissue, as a part of the endocrine system, may also have important influences on the amount of adipokines secreted either via increased size (hypertrophy) or increased numbers (hyperplasia).[29]

In the context of this complexity, we investigated the longitudinal associations of adipokine (adiponectin and leptin) levels and adiposity (overall fatness and truncal fatness), without a priori assumption on the direction of the associations, from adolescence to young adulthood among participants in a Chilean infancy cohort study. Specifically, by applying structural equation models (SEM), we simultaneously modeled the pathways through which adolescent adipokine levels may impact downstream young adulthood adiposity and the pathways through which adolescent adiposity may impact downstream young adulthood adipokine levels (**Figure 1**). In addition, since leptin resistance is closely associated with insulin resistance (IR)[30], we hypothesized that there could be heterogeneity in the association between adipokines and adiposity among those with metabolic disturbance (measured by baseline IR), and thus we further explored how these findings might differ by levels of IR status, as a proxy for baseline metabolic health status, during adolescence. Findings from this study may inform a better understanding of the complex relationships between adipokines and body fat accumulation during a critical period of the life course.

METHODS

Study Population

Study subjects were participants of the Santiago Longitudinal Study (SLS), an ongoing longitudinal infancy cohort from Santiago, Chile. SLS started as an infancy Iron Deficiency Anemia prevention trial in 1991. A total of 1,657 newborn babies from community clinics in Santiago participated in the initial preventive trial (NIH-R01HD014122). Detailed descriptions of the initial study have been presented elsewhere.[31] Follow-up exams at ages 1, 5, 10, 16, and 22 have been conducted. For the 16-year (16y; N=679) and 22-year follow-up (22y; N=1,040), data on risk factors for obesity and cardiovascular diseases (CVD) were

collected, including metabolic biomarkers.[32,33] For the current study, we defined the 'baseline' or '16y' measure as 16-year follow-up measure and the 'follow-up' or '22y' measure as 22-year follow-up measure. At baseline, a total of 634 participants had complete information for all exposure, outcome, and covariates. Among them, 590 had been followed up at 22y, and 537 had complete information for all the variables of interest. Participants whose measures of adiponectin, leptin, body fat percent (BF%), the proportion of trunk fat in % (PTF), and other covariates both at baseline and at follow-up were available were included in the current analyses (N=537). Ethical approval for all study waves was granted by the IRBs of the Institute of Nutrition and Food Technology, Universidad de Chile, the University of Michigan, and the University of California San Diego.

Measurements

Adiposity Total body fat mass and trunk fat mass at baseline and at follow-up was measured by dual X-ray absorptiometry (DEXA) (Lunar Prodigy Corp., Madison, Wisconsin, USA). BF% was calculated as 100×(total body fat mass/total body mass) to approximate overall fatness, and PTF was calculated as 100 ×(trunk fat mass/total body fat mass) to approximate central fat distribution by measuring the proportion of fat in the trunk region.

Adipokines Baseline adiponectin and leptin levels were measured using overnight fasting blood samples by enzyme-linked immunosorbent assays (ELISA) (R&D Systems, Minneapolis, MN, USA and DRG International, Inc., New Jersey, NJ, USA, respectively). Follow-up serum adiponectin and leptin levels were also measured using overnight fasting blood samples by ELISA (R&D Systems, Minneapolis, MN, USA and Diagnostic System, Webster, TX, USA, respectively).

Health-Related Factors Current alcohol drinkers at baseline were defined as those who ever used alcohol and the last alcohol use was in the past 30 days at the time of the survey. Current

smokers at baseline were defined as those who ever used cigarettes and the last cigarette use was in the past 30 days at the time of the survey. To adjust for the potential influence of participants' diet and physical activity, we used scores from self-reported questionnaires for assessing participants' dietary habits and physical activity habits at baseline, respectively. For dietary habit, the questionnaire consisted of five questions (individual scores from 0 to 2 representing low, fair, and high quality, respectively) about the number of meals per day, quality of each meal, and quality of snacks at home and at school. For physical activity, there were also five questions about sedentary time, amount of daily walking, formal/informal recreational activity. Each of these five scores was summed as overall diet quality and an overall physical activity score ranging from 0 to 10, respectively. The questionnaires were applied previously for school-aged Chilean children.[34,35] To account for potential confounding by participants' socioeconomic status (SES), a binary attained education variable indicating whether a participant completed at least 12 years of formal education compared to those who did not. For females, to adjust for the potential health impact of pregnancy, in particular with respect to adiposity, we considered information on whether a mother had ever delivered a live birth by the age of 22y.

Insulin resistance IR was measured by homeostatic model assessment of insulin resistance (HOMA-IR). HOMA-IR was calculated as [(glucose(mg/dL) × insulin(μUI/dL))/405], and IR status was defined as HOMA-IR≥2.6.[36] Overnight fasting serum glucose levels were measured with an enzymatic colorimetric assay (QCA S.A., Amposta, Spain). Overnight fasting insulin levels were measured with radioimmunoassay (RIA DCP Diagnostic Products Corporation, LA, USA).

Conceptual model of the longitudinal relationships among adiposity and adipokines

Figure 1 displays hypothesized relationships among variables. We hypothesized that BF%,

PTF, adiponectin levels, and leptin levels at the baseline exam could affect BF%, PTF, adiponectin levels, and leptin levels at follow-up. We assumed those variables were correlated within each time period (i.e., cross-sectionally) at baseline and at follow-up. Due to the substantial difference in leptin levels and BF% by sex – which has been reported in previous reports as well, we conducted sex-stratified analyses. Smoking status, alcohol drinking status, diet, and physical activity at baseline were hypothesized to be in the pathway to adiposity and adipokine levels both at baseline and follow-up. We did not account for the covariate measures at follow-up because there might be potential reverse causation between the follow-up adipokine or adiposity measures and the follow-up health-related factor measures. Since participants were captured during the lifecycle period of schooling, we relied on attained education at age 22y to capture the school-aged period. Age at baseline was assumed to affect the adiposity and adipokine levels at baseline, and time between baseline and follow-up was assumed to impact the adiposity and adipokine levels at follow-up. Pregnancy during follow-up was assumed to affect adiposity and adipokine levels at follow-up.

Statistical analyses

We utilized SEM to estimate and evaluate the longitudinal associations between adiposity (BF% and PTF) and circulating adipokines (adiponectin and leptin) levels. A series of linear models between multiple outcomes and multiple explanatory measures were simultaneously assessed in the path model. This allowed us to test each longitudinal association between these four measures at baseline and each of the four measures at follow-up. Adjusted variables were described in the previous section and in **Figure 1**. For those with leptin levels equal to or under the detection limit (1 ng/mL), we assigned values of 0.5 ng/mL (half of the detection limit).[37] All the main variables of interest (BF%, PTF, adiponectin levels, and leptin levels at baseline and follow-up) were natural log-transformed. To aid in comparing the

magnitude of associations, we present standardized estimates of exposure and outcomes from these models. In addition, we stratified by baseline IR status (considered as a proxy of metabolic health status during adolescence) to investigate potential effect modification by baseline metabolic health status on the longitudinal association between adipokines and adiposity. To formally test for interaction by sex and IR status (within each sex-stratum), we estimated four separate generalized linear regression models for the four dependent variables (BF%, PTF, adiponectin, and leptin at follow-up) and assessed the statistical significance of interaction (P-value for interaction < 0.1 was considered significant.).

As sensitivity analyses, we also conducted both sexes-combined analyses using sex-specific standardized (mean: 0, standard deviation: 1) values of natural log-transformed BF%, PTF, adiponectin levels, and leptin levels. Also, we additionally adjusted for age at menarche among females to investigate whether there was a potential confounding by developmental stages. All participants had completed their pubertal development and were at Tanner stage 5. Lastly, we considered longitudinal associations of adiposity with leptin-to-adiponectin ratio (LAR), an aggregate index of adiponectin and leptin measures which is considered an emerging marker for cardiometabolic risk prediction.[38,39]

Model fit was assessed by two different statistics – Root mean square error of approximate (RMSEA) and comparative fit index (CFI). We acknowledged that these are only a subset of model fit statistics and selected these statistics prior to analyses (well-accepted and complementary). Path models with RMSEA < 0.08[40] and CFI ≥ 0.90[41] generally indicate goodness of fit. In our case, given slight deviations from these criteria, we validated the effect estimates from SEM and those from separate generalized linear regression models for all four dependent variables. Path analyses and multiple linear regression analyses were conducted using PROC CALIS and PROC GENMOD from SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA), respectively.

RESULTS

Characteristics of participants

A total of 537 participants were included in the analysis. Baseline characteristics of the participants are reported in **Table 1** and **Table S1**. The average age at baseline and at follow-up was 16.8 (SD: 0.3) years and 22.6 (SD: 0.4) years, respectively. The average follow-up period was 5.8 (SD: 0.4) years. Approximately 49.5% (N=266) of participants were female. Substantial sex differences in leptin levels and BF% at baseline and follow-up were noted. The number of participants with IR at baseline among females was 47 (17.7%) and among males was 43 (15.9%).

Longitudinal associations among BF%, PTF, adiponectin levels, and leptin levels from adolescence to young adulthood

Table 2 (**Table S2** for full results) displays the SEM analysis results. For females, baseline BF% levels were positively associated with follow-up leptin levels [standardized effect estimate(SE): 0.58(0.06), $p < 0.0001$], and baseline PTF values were negatively associated with follow-up adiponectin levels [standardized effect estimate(SE): -0.31(0.06), $p < 0.0001$] and leptin levels [standardized effect estimate(SE): -0.15(0.06), $p = 0.009$]. In terms of the longitudinal association from adipokine to adiposity, both baseline adiponectin and leptin levels were positively associated with follow-up BF% [standardized effect estimate(SE): 0.12(0.04), p=0.006; 0.12(0.05), p=0.024, respectively], but no significant associations between baseline adipokines levels and follow-up PTF were observed. For males, baseline BF% was positively associated with follow-up leptin levels as well [standardized effect estimate(SE): 0.53(0.05), $p < 0.0001$], but baseline PTF was negatively associated with only adiponectin levels [standardized effect estimate(SE): -0.18(0.06), p =0.006]. In addition,

only baseline adiponectin levels (not leptin levels) were positively associated with subsequent BF% [standardized effect estimate(SE): 0.12(0.04), p = 0.007], whereas baseline leptin levels were negatively associated with follow-up PTF [standardized effect estimate(SE): -0.11(0.05), p =0.022]. As expected, significant interactions by sex ($p < 0.1$) were observed for the association between baseline leptin levels and follow-up BF% and between baseline leptin levels and follow-up PTF (**Table S6**). We also confirmed the findings from the SEM in four separate linear regression models (**Table S6, S7**) allowing us to consider each individual pathway in the larger SEM.

For the sex-combined analyses, baseline BF% were positively associated with follow-up leptin levels, and baseline PTF was negatively associated with both follow-up leptin and adiponectin levels (**Table S2**). In addition, baseline adiponectin levels were positively associated with subsequent BF%, while baseline leptin levels were negatively associated with subsequent PTF (**Table S2**). Also, further adjusting for age at menarche among females did not materially change the patterns of the estimated relationships (**Table S4**). The results for LAR demonstrated the positive associations between baseline BF% and follow-up LAR and between baseline PTF and follow-up LAR. However, baseline LAR was not significantly associated with follow-up BF% or PTF (except for PTF among males) (**Table S5**). Additional sensitivity analyses adjusting for smoking and drinking status at follow-up did not change the patterns of associations (**Table S8**).

Longitudinal associations among BF%, PTF, adiponectin levels, and leptin levels from adolescence to young adulthood by baseline IR status

We further investigated the longitudinal associations according to the baseline levels of IR (**Table 3**). Among the females with IR at baseline, no significant associations between baseline PTF and both adipokines at follow-up and between baseline adiponectin and follow-

up BF% were noted. However, no interaction by baseline IR status was observed among females (**Table S7**). For males, the associations between baseline BF% and follow-up leptin levels, between baseline PTF and follow-up adiponectin levels, and between baseline leptin levels and follow-up PTF were non-significant in the baseline IR group (p for interaction < 0.1 for 16y BF% → 22y leptin and 16y leptin → 22y PTF; **Table S7**). Baseline BF% was negatively associated with follow-up adiponectin levels only among the IR group.

DISCUSSION

In this study, we investigated longitudinal associations between circulating adipokine levels and adiposity measured with DEXA from adolescence to young adulthood within the Chilean infancy cohort. For both females and males, we found consistent evidence for a positive association between baseline BF% and follow-up leptin levels, a negative association between baseline PTF and follow-up adiponectin, and a positive association between baseline adiponectin and follow-up BF%. Among males, we also observed a negative association between baseline leptin levels and follow-up PTF. Among females, we observed a negative association between baseline PTF and follow-up leptin levels and a positive association between baseline leptin and follow-up BF%.

In line with the majority of cross-sectional and longitudinal[22,23] studies, we found a positive association between BF% and leptin – i.e., 16y BF% → 22y leptin and (primarily among females) 16y leptin → 22y BF%. Leptin levels are well known to be proportional to the amount of body fat,[6] and thus, our observed associations between baseline BF% with follow-up leptin levels were expected. In addition, this relationship also suggests that resistance to leptin[42] – i.e., circulating leptin cannot increase the energy expenditure or suppress appetite anymore – can begin early in the life course. Regarding the longitudinal

association between baseline leptin and subsequent BF%, as our results demonstrated a positive association for females and a negative association (though not significant) for males, previous studies of children and adolescents have found both negative[15,16] and positive[14,19,24,26] associations between baseline leptin levels and subsequent increases in adiposity (measured by fat mass or body weight). Discrepancies may be related to differences in pubertal development or the baseline obesity status of study participants. Our study investigated unique life-course effects – from post-pubertal adolescence to young adulthood. Furthermore, previous studies investigated DEXA-measured fat mass[14,19,26], fat mass index[16], or body mass index (Z-score)[15,24], while the current study focused on DEXA-measured BF%.

In addition, we observed a negative association between trunk (central) fat (i.e., PTF) and adiponectin levels, which most previous cross-sectional investigations also reported. Our results suggest that the negative association between central fat and adiponectin is driven by the influence of fat accumulation in the trunk/central region and subsequent changes in adiponectin levels. Indeed, a previous intervention study has suggested a causal effect of visceral fat on adiponectin levels among people with obesity and overweight.[43] In contrast, our results did not strongly support a longitudinal influence of adiponectin on central fat, although some studies have previously reported such effects, for example, for trunk fat mass (or percent)[44] and abdominal fat[45]. Overall, our results suggest that an accumulation of intraabdominal fat or visceral adipose tissue (VAT) may lead to a subsequent reduction in adiponectin levels, supporting a mediating role of adiponectin levels in the well-established relationship between VAT and cardiometabolic disorders (reviewed in [46]). However, as DEXA cannot confirm the exact location of fat depots[47], further studies are needed to apply accessible methods that can distinguish VAT and subcutaneous adipose tissue (SAT) in the setting of longitudinal adipokine measurements.

Previous studies of the *longitudinal* associations of baseline adiponectin with

subsequent overall adiposity have been inconsistent[17,18,20,21,25], and we report a positive longitudinal association between baseline adiponectin and follow-up BF%. In support of our findings, murine studies have reported that overexpression of adiponectin led to both increased fat mass and improved insulin sensitivity.[20] In addition, a Nurses' Health Study reported positive associations between baseline adiponectin and longitudinal weight gain among non-diabetic participants.[18] In contrast, other studies have reported inverse associations between baseline adiponectin levels and follow-up weight change.[17,48] For example, an early mouse study revealed that adiponectin administration led to sustainable weight loss.[48] These observed inconsistencies may imply that the effects of adiponectin on fat accumulation depend on where fat is deposited. Han et al (2017) demonstrated that increases in fat accumulation were related to lower baseline adiponectin levels, but only if in the abdominal visceral fat.[17] In addition, murine studies have demonstrated that overexpression of adiponectin increased adipose tissue but improved insulin sensitivity.[20] Thus, adiponectin may protect against cardiometabolic risk while increasing body fat accumulation. Also, it is possible that the transition to young adulthood is a critical period for adiponectin-associated biological changes. Moreover, adiponectin is not exclusively secreted in the adipose tissue.[49-52] Similarly, BF% is influenced by both the size of adipocytes (hypertrophy) and the numbers of adipocytes (hyperplasia); thus the association between BF% and adiponectin levels may differ by the relative influence of hypertrophy and hyperplasia in increasing BF%.[29] Unfortunately, DEXA does not facilitate such comparisons.

We also observed context-specific negative associations between baseline PTF and 22y leptin (females with normal IR level) and between baseline leptin and 22y PTF (males with normal IR level). While leptin administration in patients with lipodystrophy has been shown to decrease in trunk fat mass or ectopic fat mass (reviewed in [53]), other studies demonstrated a positive association between baseline leptin and changes in CT-measured

abdominal fat[45,54]. Inconsistencies may be related to differences in baseline health status, study population (race/ethnicity), or age. Our observed negative associations between baseline PTF and follow-up leptin levels may be related to a relatively higher concentration of VAT in the trunk region, as previous studies demonstrated leptin as a better marker of SAT compared to VAT[55]. Further investigation with accurate measures of SAT and VAT are required to validate this hypothesis.

As adipokine levels and BF% are closely tied to IR, we further stratified participants by their IR status and assessed potential heterogeneities in our findings. The association of follow-up leptin levels with baseline BF% and the association of follow-up PTF with baseline leptin levels differed by baseline IR status among males. Of note, follow-up leptin levels were strongly associated with baseline leptin levels and not with baseline BF% among males with IR at baseline. We hypothesize that leptin resistance accompanied by IR leads to greater increases in leptin levels, regardless of baseline BF%. However, due to the small number of participants within the high-risk IR stratum, the effect estimates or the interaction by IR status should be interpreted with caution.

A major strength of our study was the focus on a unique and critical developmental stage of the life course. Second, we made no assumptions about the direction of longitudinal associations between adipokines and adiposity, allowing less biased estimates. Third, the study utilized an accurate measure of adiposity – DEXA measure of BF% and PTF. Limitations include small samples sizes and our assumption that the baseline health-related behaviors would affect both baseline measures and follow-up measures. In addition, roughly a quarter of the sample at baseline had leptin values that were below the limit of detection. We accounted for values below the limit of detection using single imputation at half the detection limit, a commonly applied method, which has been shown to perform well in certain scenarios.[37] Also, due to the small number of participants with baseline IR, we may

have been underpowered to detect associations among this group, particularly for interaction by IR. Lastly, although DEXA measures of adiposity are more accurate than anthropometric measures, it is still known that DEXA underestimates BF% in lower ranges and in males and overestimates BF% in higher ranges and in females.[57]

In conclusion, we observed dynamic relationships between BF%, PTF, adiponectin levels, and leptin levels from late adolescence to young adulthood in a Hispanic/Latinos population. We observed a strong positive relationship between 16y BF% and 22y leptin levels, a negative relationship between 16y PTF and 22y adiponectin levels, and a positive relationship between 16y adiponectin and 22y BF% (except for the high-risk baseline IR group). Further efforts to elucidate causal relationships are warranted.

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Disclosure Statement

The authors have nothing to disclose.

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TABLES

Table 1. Distributions of variables at the 16-year and 22-year follow-ups of the Santiago Longitudinal Study (SLS)

	Female (N=266)		Male (N=271)	
Variable	Mean / N	SD / %	Mean / N	SD / %
Adiposity				
Body fat percent† at 16y (%)	36.2	7.4	21.9	8.9
Body fat percent at 22y (%)	40.5	6.6	29.0	7.4
Proportion of trunk fat‡† at 16y (%)	49.9	4.9	49.4	6.2
Proportion of trunk fat at 22y (%)	49.9	5.6	52.3	5.5
Adipokine				
Adiponectin at 16y (ug/mL)	12.6	5.8	10.5	4.9
Adiponectin at 22y (ug/mL)	7.4	4.7	5.7	3.6
Leptin at 16y (ng/mL)	19.0	14.2	6.1	9.2
Leptin at 22y (ng/mL)	32.8	18.6	10.6	11.1
Socio-demographic variables				
Age (16y)	16.8	0.3	16.8	0.3
Age (22y)	22.6	0.4	22.6	0.4
Follow-up period (years)	5.8	0.4	5.8	0.3
Higher education*				
Yes	238	89.5	228	84.1
No	28	10.5	43	15.9
Health-related variables at baseline (16y)				
Pregnancy between 16y and 22y**				
Yes	100	37.6	-	-
No	166	62.4	-	-
Diet habit score (0 - 10) §	5.3	1.3	5.2	1.1
Physical activity score (0 - 10) §	3.4	1.3	4.8	1.7
Smoking status at 16y				
Current smoker	59	22.2	45	16.6
Non-smoker	207	77.8	226	83.4
Alcohol drinking status at 16y				
Current drinker	44	16.5	74	27.3
Non-drinker	222	83.5	197	72.7
Metabolic health at baseline (16y)				
Fasting glucose	86.0	9.0	90.0	8.8
Fasting insulin	8.4	5.4	7.8	5.9
HOMA-IR	1.8	1.3	1.8	1.4
Insulin resistance status***				
Insulin resistance	47	17.7	43	15.9
Normal	219	82.3	228	84.1

†Body fat percent = 100 * [total fat mass (g) / body mass (g)]

‡Proportion of trunk fat = 100 * [trunk fat mass (g) / total fat mass (g)]

*Complete higher education at least 12 years of formal education (proxy for socioeconomic status)

** Measured as whether a mother had delivered a live birth by the age of 22y

***Used as a proxy of baseline metabolic health

§ Higher scores represent healthier diet quality and physical activity status, respectively.

Table 2. Longitudinal associations between adiposity and adipokine from the path analysis in participants of SLS

Path			Female[†]			Male[‡]		
Baseline (16y)		Follow-up (22y)	Effect estimate[*]	SE	p-value	Effect estimate	SE	p-value
Baseline adiposity to follow-up adipokine								
BF%	→	Adiponectin	0.0880	0.0718	0.2207	0.0462	0.0606	0.4461
	→	Leptin	0.5832	0.0623	<0.0001	0.5308	0.0525	<0.0001
PTF	→	Adiponectin	-0.3149	0.0606	<0.0001	-0.1765	0.0636	0.0055
	→	Leptin	-0.1539	0.0591	0.0092	-0.0768	0.0616	0.2124
Baseline adipokine to follow-up adiposity								
Adiponectin	→	BF%	0.1178	0.0430	0.0062	0.1153	0.0424	0.0066
	→	PTF	-0.0246	0.0481	0.6085	-0.0112	0.0423	0.7915
Leptin	→	BF%	0.1178	0.0523	0.0243	-0.0294	0.0462	0.5246
	→	PTF	0.0132	0.0586	0.8222	-0.1059	0.0462	0.0219

BF%: Body fat percent (=100×total fat mass / body mass); PTF: Proportion of trunk fat (=100×trunk fat mass / total fat mass)

[*] Standardized effect estimates; Both the exposure and the outcome in each pathway were standardized to compare the magnitude of associations.

[†] SEM fit statistics: RMSEA (90% CL) 0.0616 (0.0237, 0.0972); CFI 0.9881

[‡] SEM fit statistics: RMSEA (90% CL) 0.1190 (0.0830, 0.1579); CFI 0.9716

Table 3. Longitudinal associations between adipokines and adiposity by baseline insulin resistance status from the path analysis in participants of SLS

Female	Path			Normal at baseline *			Insulin resistance at baseline **		
	Baseline (16y)		Follow-up (22y)	Effect estimate	SE	p-value	Effect estimate	SE	p-value
	Baseline adiposity to follow-up adipokine								
	BF%	→	Adiponectin	0.0799	0.0757	0.2913	0.0377	0.1739	0.8283
		→	Leptin	0.5553	0.0685	<.0001	0.5884	0.1351	<.0001
	PTF	→	Adiponectin	-0.3623	0.0647	<.0001	0.0085	0.1445	0.9532
		→	Leptin	-0.1614	0.0662	0.0148	0.0130	0.1195	0.9134
	Baseline adipokine to follow-up adiposity								
	Adiponectin	→	BF%	0.1581	0.0480	0.0010	-0.0026	0.0927	0.9775
		→	PTF	-0.0135	0.0462	0.7704	-0.0546	0.1409	0.6982
	Leptin	→	BF%	0.1122	0.0579	0.0526	0.1740	0.1110	0.1170
		→	PTF	0.0203	0.0558	0.7156	-0.1159	0.1681	0.4905
Male	Path			Normal at baseline†			Insulin resistance at baseline‡		
	Baseline (16y)		Follow-up (22y)	Effect estimate	SE	p-value	Effect estimate	SE	p-value
	Baseline adiposity to follow-up adipokine								
	BF%	→	Adiponectin	0.0322	0.0640	0.6144	-0.7164	0.2549	0.0050
		→	Leptin	0.5267	0.0538	<.0001	-0.3638	0.2486	0.1434
	PTF	→	Adiponectin	-0.1898	0.0691	0.0060	-0.0905	0.1442	0.5302
		→	Leptin	-0.0632	0.0659	0.3374	-0.0533	0.1428	0.7090
	Baseline adipokine to follow-up adiposity								
	Adiponectin	→	BF%	0.1074	0.0477	0.0245	0.1769	0.0932	0.0579
		→	PTF	-0.0016	0.0469	0.9722	0.0938	0.1015	0.3554
	Leptin	→	BF%	-0.0342	0.0502	0.4961	-0.1147	0.1943	0.5548
		→	PTF	-0.1246	0.0494	0.0116	0.2470	0.2162	0.2532

BF%: Body fat percent (=100 × total fat mass / body mass); PTF: Proportion of trunk fat (=100 × trunk fat mass / total fat mass)

* SEM fit statistics: RMSEA (90% CL) 0.0690 (0.0278, 0.1082); CFI 0.9862

** SEM fit statistics: RMSEA (90% CL) 0.0750 (0.0000, 0.1768); CFI 0.9840

† SEM fit statistics: RMSEA (90% CL) 0.1213 (0.0817, 0.1640); CFI 0.9684

‡ SEM fit statistics: RMSEA (90% CL) 0.1465 (0.0000, 0.2577); CFI 0.9735

FIGURE LEGENDS

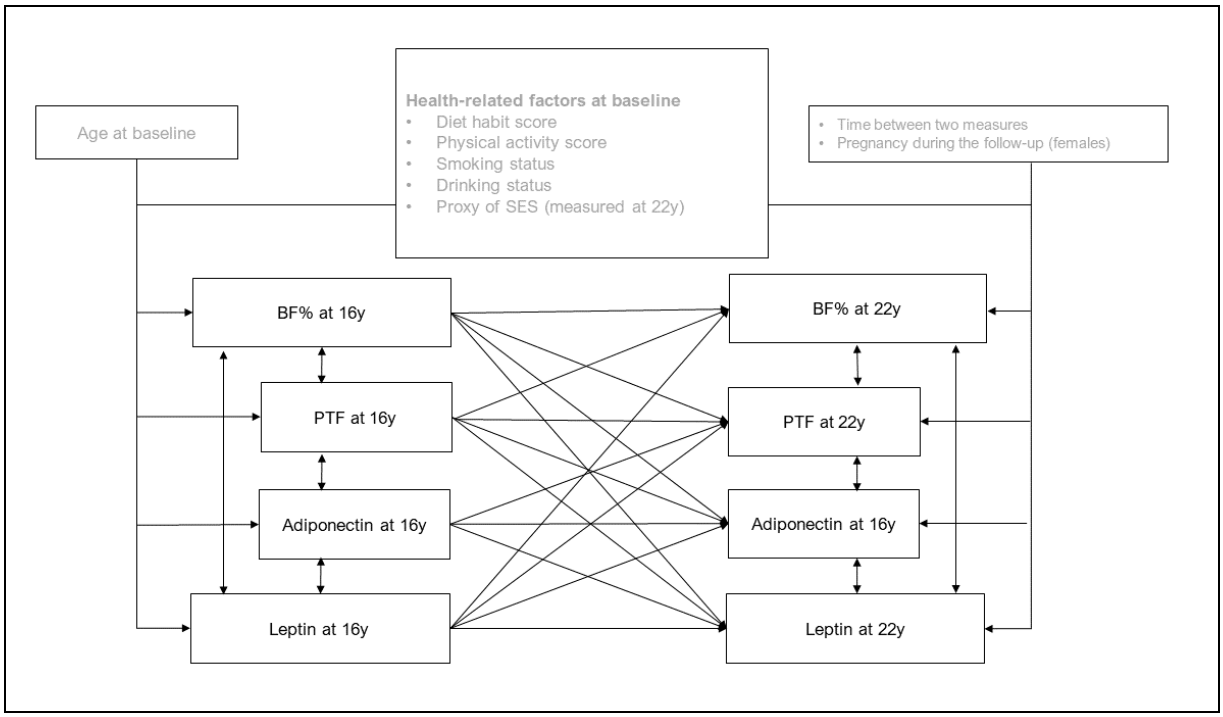

Figure 1. Conceptual diagram for the longitudinal relationships between adiposity and adipokines. We hypothesized that BF%, PTF, adiponectin, and leptin at the baseline exam could affect those measures at follow-up. We assumed those measures were correlated within each time period (bidirectional arrows). Baseline smoking, alcohol drinking, diet, physical activity, and a proxy of SES were hypothesized to be in the pathway to adiposity and adipokine levels both at baseline and follow-up. Age at baseline was assumed to affect the adiposity and adipokine levels at baseline, and time between baseline and follow-up and pregnancy during the follow-up period (for females) were assumed to impact the adiposity and adipokine levels at follow up.

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